

application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification

Please cancel the existing Sequence Listing for the above-identified application, replace it with the substitute Sequence Listing appended hereto, and insert the same at the end of the application. Applicants' attorney hereby states that the change made in the sequence listing does not include new matter. Applicants' attorney has amended the specification only to direct the entry of this corrected Sequence Listing at the end of the application and to correct sequence identification numbers in the paragraphs described below.

In accordance with 37 C.F.R. § 1.825(b), the paper copy of the Substitute Sequence Listing and the computer readable copy of the Substitute Sequence Listing submitted herewith are the same.

Please substituted the paragraph starting on page 11, line 4, with the following paragraph:

Figure 5. Alanine-scan of PTH(1-14). Shown are the bioactivities of 14 different PTH(1-14) derivatives, each having a different amino acid of the native sequence (SEQ ID. NO:73; shown at bottom of figure) replaced by alanine. Peptides were chemically synthesized, purified and tested for ability to stimulate cAMP formation in COS-7 cells

B²

... the ... and human PTH₁ receptor. Peptides were tested in duplicate (+ s.e.m.) At

B² a dose of 67 μ M. As a control, untreated cells, indicated by basal, were measured. The PTH(1-14) containing alanine at position 1 was used as the wild-type reference. Cells were stimulated for 30 minutes at 21 °C. This figure provides information relevant to bioactivities of amino acid residues in the PTH(1-9) peptide used in the invention.

Please substitute the paragraph starting on page 13, line 12, with the following paragraph:

B³ Figure 17. Nucleotide sequence (SEQ ID NO:61) and corresponding amino acid sequence (SEQ ID NO:62) of hP1R-Tether-1 (hP1R-Tether(1-9)). Made from the human PTH-1 receptor by replacing Ala24 to Arg181 with Ala1 to His9 of PTH. HK-Tether-1: Sequence ID#: E20986A1 (99nts) and its translation. Oligo to construct Tether-1 in hPTH-1 rec (HK). Join Ala-23 of rec to Val-2 of PTH(1-9)--Glyx4, --Glu-182
ctctgctgccccgtgctcagctccgcgtacgcgGtttCCGAAAtCCAGCtGAtGCACggc-
L C C P V L S S A Y A V S E I Q L M H G -
ggaggaggcgagggtgttgaccgcctgggcatgatctac (SEQ ID NO: 50)
G G G E V F D R L G M I Y . (SEQ ID NO: 55)

Please substitute the paragraph starting on page 14, line 1, with the following paragraph:

B⁴ Figure 19. Nucleotide sequence (SEQ ID NO:57) and corresponding amino acid sequence (SEQ ID NO:58) of hP1R-[R11]-Tether(1-11). Made from hTether-1 by inserting Asn10-Arg11 between His9 and first Gly of linker. Fig 19 Sequence ID#: E27309A1. hThr-Arg11: Insert Asn 10 and Arg11 into

B4 HK-Tether-1 *** Adds NSII site at Met8/His9 (ATGCAt)

CCGAAAtCCAGCtGAtGCAtAAtCGtggcggaggagcgaggtgtttg (SEQ ID NO: 69)

E I Q L M H N R G G G G E V F D (SEQ ID NO: 70)

Please substitute the paragraph starting on page 73, line 25, with the following paragraph:

B5 Depicted in Figure 11 is the chemically synthesized oligonucleotide (oligo) (#E16631A1) that was used to construct the chimeric rat PTH-1 receptor, rTether-1, which contains at its N-terminus residues (1-9) of rat PTH (A-V-S-E-I-Q-L-M-H-) (SEQ ID NO: 74) fused to Glu-182 of the receptor via a tetraglycine linker. The oligo thus encodes the rPTH(1-9) ligand sequence and four Gly residues in its central portion, and rPTH receptor residues as flanking portions. Also shown is the control oligo (E16853A1) that is similar to E16631A1 but in place of rPTH(1-9) there is the amino acid sequence (P-Y-D-V-P-D-Y-A-) (SEQ ID NO: 71) corresponding to the HA epitope tag; this will yield a receptor construct that we described previously (Luck et al., 1999 Mol.Endo , 13; 670-680).

Please substitute the paragraph starting on page 77, line 9, with the following paragraph:

B6 PTH Receptor mutagenesis and COS-7 cell expression: The pCDNA-1-based plasmid encoding the intact hPTH-1 receptor (HK-WT in reference (Schipani, E., *et al.*, *Endocrinol.* 132:2157-2165 (1993)) and herein called hP1R-WT) was used for studies in COS-7 cells. The truncated human PTH-1 receptor (hP1R-delNt) (Figure 18) was constructed from the HK-WT plasmid by oligonucleotide-directed mutagenesis (Kunkel, T.A., *Proc. Natl. Acad.*

Sci. USA 82:488-492 (1985)). This mutant receptor is deleted for residues 24 to 181 and, assuming that signal peptidase cleavage occurs between Ala²² and Tyr²³ (Nielsen, H., *et al.*, *Protein Engineering* 10:1-6 (1997)), is predicted to have Tyr²³ as the N-terminal residue joined directly to Glu¹⁸² located at or near the boundary of the first transmembrane domain. A similarly truncated rat PTH receptor was described by us previously (Luck, M., *et al.*, *Molec. Endocrinol.* 13:670-680 (1999)). The tethered human PTH-1 receptor [hP1R-Tether(1-9)] (hTether-1 in Figure 17) is based on the hP1R-delNT construct, and has PTH(1-9) and a four glycine spacer (AVSEIQLMHGGGG) (SEQ ID NO: 72) inserted between residues 23 and 182. Assuming that signal peptidase cleavage occurs between Ala²² and Tyr²³, hP1R-Tether(1-9) is predicted to have Tyr²³ as the N-terminal residue joined directly to Ala¹ of the ligand. Analogs of hP1R-Tether(1-9) were made in a similar fashion. Transient transfections of COS-7 cells were performed using DEAE-dextran and 200 ng of cesium chloride-purified plasmid DNA per well of a 24-well plate, as described previously (Bergwitz, C., *et al.*, *J. Biol. Chem.* 272:28861-28868 (1997)).

Please substitute the paragraph starting on page 78, line 16, with the following paragraph:

The study began with the construction of the targeted tethered ligand/receptor constructs, which utilized a previously reported delNT receptor as a point of departure (Luck, M., *et al.*, *Molec. Endocrinol.* 13:670-680 (1999)). This mutant receptor lacks residues 24 – 181 of the extracellular N-terminal ligand-binding domain, and is predicted to have Tyr²³ as the N-terminal residue joined directly to Glu¹⁸² following signal peptidase cleavage. In order to construct a tethered ligand/receptor construct (hTether), the following 13 amino acid

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sequence was inserted between Tyr²³ and Glu¹⁸²: Ala-Val-Ser-Glu-Ile-Gln-Leu-Met-His-(Gly)₄ (SEQ ID NO: 72). Thus, after signal peptidase cleavage, it is predicted that hP1R-Tether(1-9) should contain (C-term to N-term) the intracellular C-terminal domain, the seven transmembrane helices (and accompanying loops), a short glycine spacer and [Tyr¹]-rPTH(1-9). Other tethered ligand/receptor constructs were made in the same fashion, wherein only the sequence corresponding to rPTH(1-9) was expanded in the C-terminal direction by one or two amino acids as in hP1R-[R(1-11)] (Figure 19).

Please substitute the paragraph starting on page 83, line 11, with the following paragraph:

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Interaction between the N-terminal residues of PTH and the region of the PTH receptor containing the extracellular loops and transmembrane domains is thought to be a critical step in receptor activation. This hypothesis was evaluated by replacing the N-terminal extracellular domain of the hPTH-1 receptor with residues (1-9) of rPTH (AVSEIQLMH) (SEQ ID NO: 74) using a tetraglycine linker between His-9 and Glu-182 at the extracellular end of the first transmembrane domain to yield hP1R-Tether(1-9). Expression of hP1R-Tether(1-9) in COS-7 cells resulted in basal cAMP levels that were 4- to 5-fold higher than those seen in control cells transfected with hP1R-wildtype. Extending the ligand sequence to position-11 and including the activity-enhancing substitution of Leu-11→Arg yielded hP1R-[R¹¹]Tether-(1-11) which resulted in a 20-fold increase in basal cAMP signaling, which approached the maximum agonist-stimulated response attained by hP1R-wildtype. Alanine-scan of hP1R-[R¹¹]Tether-(1-11) revealed that Val-2, Ile-5 and

Met-8 were crucial for autoactivation. Thus, tethered ligand receptor constructs having